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NUTRITION REVIEWS

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NUTRITION REVIEWS

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SOME EARLY EXPERIENCES IN NUTRITIONAL RESEARCH

In 1920, when topics for doctorate research were being discussed with Professor Lafayette B. Mendel, I expressed an interest in pancreatic secretin. Professor Mendel said that a study of secretin would not be a good one for me to attempt at that time. Such a problem was best reserved until one had a post and was in a position to continue the work without regard for a "dateline."

Later that year, Professor Mendel returned from the "Federation Meetings" and reported hearing a paper the gist of which was the claim that the hormone pancreatic secretin was probably the same as what was being called "vitamin B." He expressed grave doubts about the validity of such an idea, and proposed that I make a similar study as the basis for my dissertation. He said that he and Dr. Osborne at the Connecticut Agricultural Experiment Station had a very potent vitamin B preparation made from yeast which I might test for possible secretin potency in dogs. He also cited a paper by a Swiss worker which suggested that "vitamin B" might be a stimulant of the salivary glands. Such were the circumstances that led to that part of my dissertation concerned with the secretion of glands. All of the experiments were negative so far as the hypothesis under investigation was concerned.

When planning the experiments involving pancreatic and salivary fistulas, it occurred to me that it would improve my dissertation if I could present in it positive proof that my various extracts containing "vitamin B" were really quite potent. Therefore some pigeons were secured and placed on a white rice diet and the typical head-retraction picture of advanced vitamin B deficiency was readily obtained. Injection of any of these extracts into the pectoral muscle resulted in the usual striking relief of these symptoms, in some cases within about an hour. As a result I have always felt I understood in part at least how Dr. R. R. Williams must have felt when he observed the effect of his early rice polishings extracts on Philippine babies exhibiting infantile beriberi.

Professor Mendel then suggested that I try feeding some artificial food mixtures to dogs as a follow-up of some experiments done the previous year by Walter Karr who had just graduated. Karr's observation of a loss of appetite for the artificial ration was confirmed and extended. In later years, with the help of many students and research fellows, all of this work was utilized for the development of a method for successfully feeding artificial mixtures to dogs being used in nitrogen metabolism experiments and other studies in which it was particularly important that the dog voluntarily eat a constant amount of the experimental diet daily. One of my colleagues later reported to me that by this technic he had maintained a dog essentially in nitrogen equilibrium for about two years, the interruptions being due merely to some test source of nitrogen, a purine for example, being fed; when its nitrogen had been eliminated, the dog was back again in nitrogen equilibrium!

This loss-of-appetite phenomenon exhibited by our dogs naturally led to a search for an explanation. One possibility was that the vitamin deficiency acted in some way on the mechanism underlying the gastric "hunger contractions." To test this idea it was necessary to produce gastric fistulas and study the hunger contractions during dietary regimens of interest. Although in the course of time the vitamin deficiency was observed to produce changes in the contractions, the urge to eat was restored

very promptly when the vitamin was given, whereas a long period of recovery was required before the hunger contractions had returned to normal. It was therefore concluded that the cause of this peculiar loss of appetite lay somewhere else than in the gastric mechanism. It looked as though experiments involving the brain might give the answer.

Quite early in my work I became impressed with the relatively greater amount of vitamin B needed by the rat as compared with the dog. Efforts of many biochemists to isolate the factor (now known to be thiamine) had led to failure, the active principle being lost somewhere along the line in the attempted isolation. We now know why this loss occurred. Back in the twenties, however, it looked as though this failure might stay with us for a long time. Therefore, I tried to measure the amount of the vitamin needed for as many different species of laboratory animals as possible, and enlisted the help of several students and postdoctorate fellows in this project.

Data were obtained from mice, rats, pigeons and dogs. One day I happened to come into my office and saw a graph from an unusual angle; what I saw suggested a new idea. This was followed up and eventually led to the formula which was published and from which a guess might be made as to the requirement of an "average man."

To test this idea, data on the vitamin B content of dietaries used throughout the world which were associated with human beriberi were collected. Similar data were also gathered together pertaining to diets *not* associated with this disease. The requirement formula was then applied to all of these data. I think the results turned out to be more favorable to my general views than I had a right to hope at the time. These studies were published in a monograph which appeared in 1934. This was just before Williams and others definitely announced the elucidation of the structure of vitamin B. With the vitamin now available in pure crystalline form, it was possible to make more precise studies than the indirect one I had made and published in my monograph. On the basis of the international unit for the vitamin then in use, and its equivalent in pure vitamin, it was my guess at that time that the daily requirement for an "average man" should be fairly close to one milligram.

Because of some criticisms which were received, it is appropriate to mention that this formula was never given a status higher than that of a "first approximation." The fact that the formula cannot be applied too rigidly over the complete range of body size in a given species tells us that factors other than the ones taken into consideration must also be operating.

When carrying out experiments involving the feeding of artificial diets to dogs I became impressed with the need for a better formula by which to estimate the body's surface area. With the help of Dr. D. Drabkin, then a National Research Council Fellow in our laboratory, measurements of surface area were made on 7 dogs ranging from 3.3 to 33 kg. in body weight. The data thus obtained were used to develop formulas based on the four ideas found recorded in the literature.

The best formula was a new one of our own which included a factor we called the "nutritive index." This index was intended to afford some correction in the Meeh-Rubner type of expression for the degree of fatness or leanness characteristic of the individual. This formula was then applied to dogs which were on feeding experiments at that time, the value of the *nutritive index* being determined for each individual animal.

In these particular experiments each dog was offered daily more food than it really needed and a record was kept of the amounts actually eaten over periods of several months. At first every animal ate all of the food offered. Soon, however, the daily intakes began to fluctuate, more food being eaten on cold days, less on warm days; the body weight, however, remained quite

constant, fluctuating between quite narrow limits. Although different diets were used, some high, some low in content of protein and other dietary factors, the factor which was eaten in most constant amount when all the diets were considered was calories. What was particularly surprising was the fact that the value of our "nutritive index" (N_i) for each animal was almost exactly the same. In other words, each animal, when given a chance under our conditions of cage life, adjusted its intake of calories so as to bring itself to the state or degree of fatness or thinness represented by the value 0.30 of our "nutritive index" ($Weight_{gm}^{1/3}/Length_{cm}$).

This finding was utilized in a practical way by many of our graduate students when performing metabolism experiments on dogs in which it was desired that the animals be in what might be called a comparable state of nutrition. Having selected an animal otherwise desirable for the study, the first thing to be done was to determine the value of its "nutritive index." Then, during a preliminary period the animal was fed more or less food as necessary to yield finally a N_i value slightly under the "adjustment value" of 0.30—about 0.285 or 0.29—slightly on the side of thinness. As a result, (1) each dog had a "normal urge to eat" every bit of the food offered, one of the important objectives in every metabolism experiment; and (2) each dog was in practically the same state of fatness or leanness, making it comparable with the other animals in the group so far as "nutritive state" is concerned.

Since that time considerable interest in this problem of degree of fatness or thinness has centered around possible significance of the body's specific gravity (work of Behnke and others, for example). Therefore, more recently I have been studying the possible significance of our old "nutritive index" or some improvement of it in relation to the problem of "lean body mass." For this work I have been furnished with much valuable data by Dr. Behnke and his associates. It is too early for a report on this project. It is pertinent, however, to point out that studies such as these could conceivably lead to the perfection of relatively simple technics by which clinicians, utilizing certain easy measurements and then appropriate formulas or tables, could quickly estimate and express in numerical terms much more precisely than is possible now the "nutritive index" or nutritive state of the individual. The possibilities are sufficiently intriguing to serve as a good stimulant for this line of effort which, if successful, would mean that these earlier studies with dogs had been of some real eventual use in human nutrition.

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INHIBITION OF GASTRIC HUNGER BY GLUCAGON

The mechanism by which higher animals regulate food intake in proportion to the metabolic requirements of the organism remains obscure. The controlling role of the blood sugar or, probably, the level of carbohydrate utilization has been championed in recent years by J. Mayer and his associates (*see Nutrition Reviews* **10,** *280 (1952)*; **11,** *55 (1953)*). In keeping with this theory is the old observation of E. Bulatao and A. J. Carlson (*Am. J. Physiol.* **69,** *107 (1924)*) that the intravenous injection of glucose inhibited gastric contractions in the fasting dog. The experience of other workers in similar experiments with glucose, particularly in man, have, however, been contradictory.

The availability of glucagon, a pancreatic factor which causes hyperglycemia by stimulating glycogenolysis (*Nutrition Re-*

views **12,** *132 (1954)*), has permitted a new approach to the study of hunger cramps. A. J. Stunkard, T. B. Van Itallie, and B. B. Reis (*Proc. Soc. Exp. Biol. Med.* **89,** *258 (1955)*) have reported their findings on the effect of glucagon on the gastric contractions of 7 normal human beings. The subjects were studied in the fasting state, contractions were recorded by an intragastric balloon, and frequent samples of capillary and venous blood were taken for sugar determinations.

Within three minutes after the intravenous injection of 0.5 to 2.0 mg. of glucagon there was a complete abolition of hunger contractions. The subjects also stated that the sensation of hunger disappeared or decreased soon thereafter. The capillary blood sugar rose promptly after the administration of glucagon and by thirty minutes the level averaged 77 mg. per cent greater than the fasting level. During this period the arteriovenous difference increased from 2.4 to about 18 mg. per cent. In 6 of the 7 experiments gastric contractions returned after one or two hours. At the time contractions occurred the blood sugar was still elevated an average of 63 mg. per cent over the control level, but the capillary venous difference averaged 6 mg. per cent.

The authors, in explaining the consistency of these results as compared to negative reports in the literature of studies in which glucose had been injected intravenously, consider four possibilities: (1) The absolute level of *blood glucose* does not seem to be of great importance because the level of blood glucose observed in these experiments was actually less than that obtained by other investigators. (2) The rise in *blood sugar or glucose gradient* likewise is greater after glucose administration. (3) The rate of *glucose utilization* is believed by the authors to be the most important factor in the inhibition of hunger. Increased arteriovenous differences have been reported after glucagon administration by T. B. Van Itallie, M. C. Morgan, and L. B. Dotti (*J. Clin. Endocrinol. Metab.* **15,** *28 (1955)*) and by H. Elrick and co-workers (*J. Clin. Invest.* **34,** *932 (1955)*). The relatively high arteriovenous differences observed in these experiments are taken as evidence of increased peripheral utilization of glucose. (4) A *direct effect of glucagon* on the mechanism producing hunger contractions not involving carbohydrate metabolism might be postulated. Little support for such a view can be offered.

Stunkard and his co-workers have apparently demonstrated the inhibition of hunger contractions in man by the administration of glucagon. The implication that glucagon does not act solely by elevating the blood sugar cannot be considered established. The experiments were not suitably designed to provide critical information on this point. In particular it would seem important to study the effect of glucose and glucagon on the same subjects and under the same experimental conditions so that a direct comparison of arteriovenous differences with comparable blood sugar levels would be available.

The ability of glucagon to inhibit hunger contractions cannot yet be considered a physiologic function of this substance in view of the scanty information concerning the hormonal action of this substance.

SCURVY IN AFRICANS

The signs and symptoms of scurvy have long been known, and one might be excused for thinking that a study of scurvy in South African Bantus would be unlikely to yield much fresh information, but a report by H. Grusin and P. S. Kincaid-Smith (*Am. J. Clin. Nutrition* **2,** *323 (1954)*) shows such a belief to be erroneous, and provides yet another example of how a well-known and often-described clinical condition may still have some surprises in store for the astute observer.

These workers made a study of scurvy in 29 males (laborers) and one female (a housewife) 30 to 60 years of age. The duration of symptoms was one to four weeks in 20 of these patients, two to three months in 7, and two to three years in 3. Scurvy developed "inexplicably" in some of these patients although their diet and work had remained essentially the same for years. The diet consisted mainly of maize porridge, bread, and some meat and cooked vegetables. The ascorbic acid in this diet came from the meat and vegetables, which were cooked for long periods. A control group of 100 nonscorbutic subjects "claimed to eat less porridge but more meat and vegetables." The diagnosis was confirmed in most of the patients "by biopsy evidence of recent spontaneous hemorrhage into muscle, subcutaneous tissue, or skin which responded rapidly to the administration of vitamin C."

Hemorrhage in the muscles of the lower limbs was found in every patient and pain in the legs was the most common complaint. Twenty-four patients had "a general bleeding tendency and/or hypertrophic gums, but in five, isolated hemorrhage in the leg muscles was the main sign of the disease." The leg hemorrhages were bilateral in 20 patients and unilateral in the rest. The calf, the posterior aspect of the thigh, the popliteal region, or the tissues around the ankle were the usual places for the hemorrhage to occur, the lesion usually being localized and well defined. Over the affected area the skin was darkly stained or edematous and brawny. The muscle hemorrhage was usually absorbed after four to six weeks, but chronic tissue changes occurred in some cases, most commonly around the ankle. These chronic changes included persistence of the pigmentation and a tight binding down of the skin and subcutaneous tissue so that natural markings were obscured, joint movement restricted, and "the ankle and foot took on a cadaveric appearance." The pigmented socklike areas of adherent skin were noticeable for as long as six months after treatment. In 2 patients "extensive chronic changes . . . producing a picture reminiscent of localized scleroderma" were found; in each case, the involved leg had been affected by scurvy some two to three years earlier. Although various other conditions, for example, edema, muscle abscesses, osteoporosis and anemia, were found in this group of patients, the commonest findings were the leg lesions already described, and generalized bleeding and/or hypertrophic gums. The authors suggest that in Africans scurvy may be responsible for the occasional isolated hemorrhage from bowel, kidney, or serous cavities that is occasionally and inexplicably found at autopsy.

The chronic leg lesions described by Grusin and Kincaid-Smith do not seem to have been described before in cases of acute scurvy, and may thus be peculiar to the scorbutic African. However, diseases like scurvy tend to be democratic in their manifestations, and why one race should have these chronic leg lesions is difficult to explain unless, perhaps, some unknown local factors are involved. Meantime, however, it would seem worthwhile to look for these leg lesions in people of other races, and particularly in those cases where local dietary habits suggest the possibility of the occurrence of scurvy.

DIABETES, PANTOTHENIC ACID AND ADRENAL FUNCTION

The increasing incidence of late vascular complications of diabetes presents a great challenge to medical research. The recognition that capillary aneurysm in the fundus of the eyes is an early and almost pathognomonic sign of one type of diabetic vascular disease which may affect not only the eye but the kidney and other organs as well has materially aided the clinical investigator. B. Becker and his associates have

summarized their clinical investigations of this condition (*Diabetes* **3,** *175 (1954)*).

Several lines of evidence are marshalled to implicate a dysfunction of the adrenal cortex as an etiologic factor in diabetic retinopathy. The administration of ACTH, 25 mg. intramuscularly, revealed an interesting difference between diabetic patients with and without retinopathy. It was observed that the eosinopenic response was frequently inadequate in the uncomplicated cases, whereas it was almost always "normal" in the patients with retinopathy. Of 11 of the patients with uncomplicated diabetes who failed to have a "normal" response to ACTH the administration of large doses of pantothenic acid was apparently successful in producing normal eosinopenic responses to intramuscular ACTH in 8. On the basis of this finding the authors concluded that "the defective adrenal cortical function of some diabetics can be attributed to a relative deficiency of pantothenate."

To gain further information concerning the state of pantothenic acid metabolism in a group of 33 diabetic patients and 43 normal control subjects, the ability to acetylate sulfadiazine was measured (G. D. Maengwyn-Davies, B. Becker, D. A. Rosen, and J. S. Friedenwald, *Bull. Johns Hopkins Hosp.* **96,** *150 (1955)*). The principle of this procedure is that acetylation of sulfonamides and other substances requires coenzyme A which contains pantothenic acid. Presumably if any deficiency of pantothenic acid existed the ability to acetylate sulfadiazine would be impaired. One of the biochemical defects observed by W. B. Bean and R. E. Hodges (*see Nutrition Reviews* **13,** *36 (1955)*) in pantothenic acid deficiency in man induced by omega-methyl pantothenate was an impaired ability to acetylate para-aminobenzoic acid. Contrary to expectation, both the diabetic patients with and those without retinopathy acetylated slightly but significantly more of the test dose than the normal subjects. This short report does not make clear whether the diabetic subjects were receiving insulin at the time of the study or were well controlled on diet. It would seem that the result might have been influenced by the state of carbohydrate metabolism at the time of the study. It may be recalled that F. C. Charalampous and D. M. Hegsted (*J. Biol. Chem.* **180,** *623 (1949)*; *Nutrition Reviews* **13,** *74 (1955)*) showed that diabetic rats had a greatly reduced ability to acetylate amines. After treatment with insulin the acetylating ability returned to normal but pantothenic acid was not effective.

In attempting to reconcile the finding of normal acetylation with the theory that some diabetic patients have abnormal pantothenate metabolism, Becker *et al.* (*loc. cit.*) cite observations on pantothenate-deficient rats which showed that the adrenal cortex was depleted of coenzyme A before the activity in the liver was impaired. It was postulated that some of the diabetic patients might represent such marginal deficiency.

The attempts by this group of workers to find metabolic differences between patients having diabetes with and without complications is welcomed by students of clinical metabolism. Some of the endocrine and nutritional differences which they have found may be of eventual significance. These observations cannot be looked upon at the present time as more than suggestive leads for further research. The evidence for an abnormality in pantothenate metabolism is not satisfactory by any rigid standards. Endocrinologists would have the greatest reservation about the use of the eosinopenic response after intramuscular administration of ACTH as a quantitative test of adrenal function. It would seem advisable to perform similar studies using modern methods of measuring adrenal cortical steriods in urine and plasma before and after intravenous administration of ACTH. It is hoped that further work on this important topic will be forthcoming.

PLASMA AND WHITE CELL ASCORBIC ACID IN LEUKEMIA AND ALLIED CONDITIONS

The possibility of utilizing the white blood cell as an easily available source of biopsy material representative of the general cell mass has come increasingly into focus. Extensive study of both cellular constituents and enzyme systems of the white cell in a variety of leukopathies has recently been summarized by W. N. Valentine (*Ann. Rev. Med.* **6,** *77 (1955)*).

It was shown by O. H. Lowry, O. A. Bessey, M. J. Brock, and J. A. Lopez (*J. Biol. Chem.* **166,** *111 (1946)*) that the white cell ascorbic acid content reflected the total body stores of ascorbic acid. These conclusions were derived from studies of patients with known low intakes before and after repletion with ascorbic acid. O. Bodansky, F. Wroblewski, and B. Markardt (*Cancer* **5,** *678 (1952)*) compared the plasma and white cell ascorbic acid levels among groups of normal subjects, patients with cancer, and patients with chronic noncancerous disease. Both the plasma and white cell ascorbic acid values were significantly lower in the two groups with disease. There were no statistically significant differences between these two groups, however.

A similar study, recently performed by A. L. Waldo and R. E. Zipf (*Cancer* **8,** *187 (1955)*), compared plasma and white cell ascorbic acid levels in a group of leukemic patients with those of patients with other hematologic disorders (reticulum cell sarcoma, Hodgkin's disease, multiple myeloma). Significant depressions from normal levels of ascorbic acid in both cells and plasma were noted in these groups. There was no difference between these two groups, however.

When a remission was achieved in any of the above diseases with cortisone or ACTH, plasma ascorbic acid levels fell to zero. A remission with cytotoxic agents, such as the nitrogen mustards or x-ray, was associated with a rise in plasma ascorbic acid.

Unfortunately, it cannot be ascertained from the above data whether these decreased levels reflect accelerated utilization by cancer tissue, previous depletion due to inadequate intake, or a combination of these factors. It is known that the need for ascorbic acid is increased in a variety of hypermetabolic states, such as hyperthyroidism and artificially induced fever. Administration of ACTH has also been shown to cause a diuresis of ascorbic acid. An approach to this problem was made by A. H. Minor and M. A. Ramirez (*Cancer Research* **2,** *509 (1942)*), who studied a group of patients with cancer and with chronic disease. They saturated the patients with high intakes of ascorbic acid and then determined the utilization as the difference between the intake and output of ascorbic acid. It was found that the cancer patients remained in greater positive balance, suggesting increased utilization.

More definitive answers regarding the utilization of ascorbic acid in cancer will be available when a combination of intake-output balance and plasma-white cell levels is determined simultaneously.

IODINE DEFICIENCY AND THE RICE DIET

Iodine deficiency is relatively rare in this country because of the widespread practice of iodination of salt and the nationwide transportation of foodstuffs from iodine-rich areas to iodine-poor areas. Endemic goiter continues to be a medical problem in other sections of the world. Goitrous individuals in Mendoza, Argentina, have

been studied by J. B. Stanbury *et al.* (*J. Clin. Endocrinol. Metab.* **12,** *191* (*1952*); *Nutrition Reviews* **10,** *167* (*1952*)), using modern isotopic methods. Increased thyroid avidity for radioactive iodine in the presence of normal metabolism was demonstrated in many such patients. The uptake values observed were frequently in the range which would be considered diagnostic of hyperthyroidism in other parts of the world.

The question frequently arises whether restrictive therapeutic diets might be so low in iodine that interpretation of tracer studies with radioiodine might be affected. G. A. Bishopric, N. H. Garrett, and W. M. Nicholson (*J. Clin. Endocrinol. Metab.* **15,** *592* (*1955*)) have presented observations on this problem with patients eating the Kempner rice diet, a form of therapy for hypertension (*see Nutrition Reviews* **6,** *293* (*1948*); **7,** *257* (*1949*); **8,** *304* (*1950*); **9,** *70, 73* (*1951*); **10,** *5, 46* (*1952*)).

Twenty patients with hypertension were studied on the Kempner regimen. They ate the "basic" diet of rice, fruit (without salt or preservatives), sugar and honey for three months. After this period gradual additions of other foods were made, depending on the therapeutic response. These additions were 2 to 14 ounces of lean meat per week, 2 to 6 ounces of potato a day, and small amounts of vegetables and legumes. The authors estimated that the "basic" diet contained 8 to 10 micrograms of iodine daily and the "modified" diet contained 20 to 25 micrograms per day. The minimum daily requirement for iodine is not definitely known but has been estimated by other workers to be between 15 and 40 micrograms per day. The radioiodine uptake of the thyroid measured twenty-four hours after a dose of I^{131} was greater than 46 per cent in 10 of the 20 patients. In 3 patients the uptake was greater than 80 per cent. Patients who had been on the rice diet for a period of time longer than three months had a greater likelihood of exhibiting increased thyroid uptake of I^{131}. For comparison, of 125 euthyroid (normal) individuals not on the rice diet only 2 had radioiodine uptakes greater than 46 per cent.

Thyroid enlargement was not noticed in any of the hypertensive subjects on the rice diet and no evidence of hypothyroidism is mentioned. Apparently the iodine intake was not deficient enough or the deficiency of long enough duration to induce goiter formation.

The possibility that other components of the diet might have inhibited normal iodine utilization is not likely. The dietary goitrogenic factors act by blocking either iodide trapping or organic binding. Uptakes when measured at twenty-four hours after the administration of tracer I^{131} would be depressed rather than increased by such agents.

It would be of considerable practical importance to clinicians to know the extent to which reduction of dietary salt intake on the various low salt diets alters the reliability of the radioiodine test. Statements in the literature on this point are contradictory. On the basis of present evidence it would be wise not to place too great importance on elevated thyroid uptake of I^{131} in patients who have been on rigid salt restriction for more than three months. In such cases the measurement of the serum protein-bound iodine provides a more reliable index of the amount of thyroid hormone available to the tissues.

WHITE-MUSCLE DISEASE IN LAMBS AND CALVES

White-muscle disease in lambs and beef calves has been recognized as a disease of economic importance to the livestock industry in Oregon for many years. In a recent paper O. H. Muth of the Oregon Agricultural Experiment Station, Corvallis,

has summarized the extensive field investigations and some accompanying laboratory studies that have been conducted over the past fifteen years (*J. Am. Vet. Med. Assn.* **126,** *355 (1955)*). This report is of particular interest since the white-muscle disease encountered in Oregon appears to represent a nutritional disturbance, yet attempts to control the disease with tocopherols have been largely unsuccessful.

Muth reports that the disease appears most consistently in the south central part of the state. It occurs throughout the year and appears particularly in month-old calves whose dams have spent the previous six months on good irrigated pasture. There have been instances where 50 per cent or more of lambs in a flock have been affected. In some small cattle herds where grazing is restricted to small irrigated areas all calves have been observed to have the condition.

Symptoms of the disease include muscular weakness and difficulty in moving. Where heart muscle is severely affected, labored breathing is seen and often death occurs within a few hours after such symptoms appear. Skeletal muscle injury is most common in lambs, and many affected calves exhibit signs of heart injury. Microscopically the muscles of affected animals reveal white striations, muscle fiber degeneration, hemorrhage and edema. Generally skeletal muscle lesions occur bisymmetrically (on both sides of the body). Heart lesions include white plaques on the endocardium, giving the appearance of a white enamel-like lining to the organ. Complete replacement of muscle fibers in the heart with some calcification of the supporting connective tissue is also seen.

In an attempt to relate the white-muscle disease to the ration, Muth observed that the disease is most generally seen where modern agronomic practices are in use and where excellent forage, usually of legume types grown under irrigation, serves as the principal feed source. In sheep the disease has been associated with the feeding of legume hays to ewes during the winter and before lambing. The disease has not been seen in dairy calves when the dams are fed concentrate mixtures for high milk production. It has, however, appeared in dairy calves when the dams were maintained in a manner similar to beef animals in the affected area.

In order to evaluate tocopherols as supplements to the available feed supply, a total of 280 ewes on ranches where the trouble was common were divided into two lots. The ewes in lot 1 were fed alfalfa hay, ladino clover straw and cubes containing 875 pounds of oats, 875 pounds of clover-seed screenings and 250 pounds of molasses. The ewes of lot 2 received the same ration, but the cubes were supplemented with 300 mg. of D-alpha tocopherol acetate per pound. The cubes were fed at the rate of one-half pound per head daily for thirty-one days prior to lambing. Losses attributable to white-muscle disease accounted for 13 out of the 103 lambs in lot 1 and 15 of the 111 lambs in lot 2. Supplementation of the feed with 150 mg. of tocopherol acetate per head daily prior to lambing had no apparent effect on the occurrence of white-muscle disease.

Of significance was the apparent effect of previous nutritional history. All but 3 of the 28 lambs that died with white-muscle disease were from ewes that had been on irrigated pasture until the winter feeding period. Only 3 of 94 lambs died from the disease when the ewes had been pastured on open nonirrigated range before the winter feeding period. This, coupled with the failure of ranch operators to observe any real benefit from administered tocopherols in affected animals, has led Muth to believe that the white-muscle disease affecting calves and lambs in Oregon is not the same condition as that occurring in poorly nourished beef calves in Scotland, described by K. L. Blaxter (*Vet. Record* **65,** *835 (1953)*). It is of interest that stiff-lamb disease, a condition apparently similar to white-muscle disease, has been

reported from New Zealand in lambs maintained on legume pastures (W. J. Hartley, *New Zealand Vet. J.* **1,** *137 (1953)*).

From these extensive field observations Muth is of the opinion that modification in forage plants resulting from rapid or rank growth under irrigation conditions may be an important contributing factor relative to the disease and that leguminous plants may be the most serious offenders. One is tempted to speculate whether a disturbed calcium: phosphorus ratio might be a contributing factor since leguminous plants appear to be implicated. Legumes often contain five to six times as much calcium as phosphorus. It is also pertinent that the disease as it occurs naturally in Oregon differs from the experimentally produced disease in that in the latter calcification of the muscle is not seen (*Nutrition Reviews* **11,** *241 (1953)*). The two disease conditions further appear to differ in that experimentally induced vitamin E deficiency in calves often involves discoloration of body fat, and tetany.

RIBOFLAVIN IN SWINE NUTRITION

Relatively few studies on riboflavin have been made with swine since the discovery and widespread use of vitamin B_{12} and antibiotics in animal rations. In the few studies available there has not been good agreement in the interpretation of the results or in the values obtained for the riboflavin requirement of growing pigs (*see Nutrition Reviews* **3,** *141 (1945)*; **8,** *336 (1950)*, and references cited by S. W. Terrill and co-workers, *J. Animal Sci.* **14,** *593 (1955)*). Most of these discrepancies may be explained by differences in experimental conditions, including age and breed of pig, types of ration, and, possibly, environment.

Studies with the "suckling" pig (less than 4 to 5 weeks old) were made by R. M. Forbes and W. T. Haines (*J. Nutrition* **47,** *411 (1952)*). They reported that the riboflavin requirement of the baby pig fed a synthetic milk diet was between 0.68 and 0.91 mg. per pound of dry matter in the food. A modified paired-feeding method for equalizing intake was utilized. Studies were made of the hemoglobin, red and white cell counts, and differential counts at intervals during these experiments. No significant differences were found in the blood. This is contrary to the studies of H. H. Mitchell, B. C. Johnson, T. S. Hamilton, and Haines (*see Nutrition Reviews* **8,** *336 (1950)*) who reported that the per cent of neutrophils in the blood was a sensitive index of the riboflavin adequacy of the diet of weanling pigs.

E. R. Miller, R. L. Johnston, J. A. Hoefer, and R. W. Luecke (*J. Nutrition* **52,** *405 (1954)*) reported that the suckling pig required approximately 1.36 mg. of riboflavin per pound of feed. A synthetic milk diet low in riboflavin was used. Detailed studies of deficiency signs were presented. These included poor growth, loss of appetite, a heavy sebaceous exudate about the eyes and ears, cataracts in the lenses, and various histologic changes. The authors stated that genetic and other experimental factors might explain the higher riboflavin requirement as compared with that reported by Forbes and Haines (*loc. cit.*). W. P. Lehrer and A. C. Wiese (*J. Animal Sci.* **11,** *244 (1952)*), in a study with 8 baby pigs, suggested that the riboflavin requirement was near 3.0 mg. daily per 100 pounds of body weight (equivalent to about 1 mg. per pound of feed).

Studies with the growing pig indicate a lower riboflavin requirement than that for the younger suckling pig. C. O. Miller and N. R. Ellis (*J. Animal Sci.* **10,** *807 (1951)*) used practical diets containing low levels of riboflavin. They concluded that the level of riboflavin required was between 0.55 and 0.83 mg. per pound of diet.

A total of 65 growing pigs fed semisynthetic diets in three separate experiments were used in riboflavin studies by Terrill and co-workers (*loc. cit.*). The pigs were reared on wire screen-bottomed cages and started on experiment after a one- or two-week depletion period. Signs of riboflavin deficiency, which appeared within a few weeks, included poor growth, lowered feed consumption, coarse hair coats, rough skins, and increased irritability and hyperactivity, confirming earlier findings. In addition, the deficient pigs had a considerable degree of scours after the first two weeks of the test. No differences were seen in the blood picture of the deficient pigs as compared with the positive control pigs, confirming the studies of Forbes and Haines (*loc. cit.*).

Histologic studies of tissues of deficient pigs showed "quite fatty" livers in 4 out of 6 pigs, although no actual values of liver fat were reported. Ovaries of 3 deficient pigs showed collapsed follicles and degenerating ova. Other observations included debris in the Bowman's capsules and tubules of the kidneys, and erosion of the villi of the intestines with a heavy infiltration of lymphocytes into the epithelium. Histologic studies with pair-fed pigs were not presented.

All signs of deficiency were prevented by the addition of sufficient crystalline riboflavin to the diet. A level of 0.65 mg. of riboflavin per pound of diet was adequate at the environmental temperature used (an average of 53° F.). The next lowest level of riboflavin tested was 0.4 mg., which was clearly suboptimal. Tests with vitamin B_{12} and chlortetracycline showed that these substances had no significant effect on the riboflavin requirement.

It is interesting that cataract formation was not reported in the two studies with growing pigs in spite of its occurrence in riboflavin-deficient suckling pigs, as mentioned above.

The dietary riboflavin requirement for reproduction of swine has been studied by C. O. Miller, N. R. Ellis, J. W. Stevenson, and R. Davey (*J. Nutrition* **51,** *163 (1953)*). A total of 26 sows was fed practical rations containing four different levels of riboflavin (0.55, 0.83, 1.25, and 1.65 mg. per pound of feed). The lowest level, 0.55 mg., proved inadequate for reproduction, resulting in death of 3 out of 7 sows and in lack of ability to conceive in 3 other sows. The one sow which conceived farrowed an apparently normal litter, but the pigs survived less than forty-eight hours. Sows receiving the 0.83 mg. level of riboflavin showed symptoms of deficiency after the second litters were produced (reduction in birth weight of pigs, reduced viability, and poor growth of suckling pigs). Normal reproduction was obtained with the addition of 1.25 mg. of riboflavin per pound of diet.

Additional studies with riboflavin in swine nutrition are needed in order to "pin point" accurately the riboflavin requirement of the pig and to determine factors affecting this requirement. Studies such as these reviewed, although necessarily preliminary, are examples of the many careful experiments necessary in order to establish nutritional requirements of farm animals. Also, such studies contribute to a better understanding of the role of riboflavin in animal nutrition and health.

PROLONGED SUBOPTIMUM CAROTENE INTAKE IN DAIRY COWS

Blood carotene and vitamin A values are widely used as measures of the carotene and vitamin A nutritional status of livestock. It is generally agreed that liver vitamin A concentration is a better indicator of intake of vitamin A and carotene than is blood vitamin A (P. R. Frey, R. Jensen, and W. E. Connell, *J. Nutrition* **34,** *421 (1947)*;

J. W. Thomas and L. A. Moore, *J. Dairy Sci.* **35,** *687 (1952)*).

The development of the liver biopsy technic in cattle has facilitated the use of liver vitamin A concentration data in assessing nutritional status of the animals with respect to this vitamin (L. Seghetti and H. Marsh, *Am. J. Vet. Res.* **14,** *9 (1953)*; C. K. Whitehair, D. R. Peterson, W. J. Van Arsdell, and O. O. Thomas, *J. Am. Vet. Med. Assn.* **121,** *285 (1952)*). The use of the biopsy technic has indicated that less vitamin A is stored in the liver during lactation in dairy cows consuming 330 micrograms of carotene per pound of body weight than in animals receiving one-fifth this level during gestation (nonlactating) (F. H. Baker, R. MacVicar, L. S. Pope, and Whitehair, *Proc. Soc. Exp. Biol. Med.* **83,** *571 (1953)*).

An interesting report from the Oregon Agricultural Experiment Station, Corvallis, deals with the metabolism of carotene and vitamin A in dairy cattle that had been subjected to suboptimum carotene intakes while in utero and subsequently through two or three lactations (J. H. Byers, P. H. Weswig, J. F. Bone, and I. R. Jones, *J. Dairy Sci.* **38,** *657 (1955)*). Eight cows were used of which 5 were from mothers that had received normal rations. The dams of the other 3 cows received 50 micrograms of carotene per kilogram of body weight during the last four, five and eight months of gestation. Blood, liver biopsy and milk samples were analyzed for vitamin A at intervals of about one month.

Responses to carotene and vitamin A were studied by supplying the former in varying amounts as a carotene-rich oil to the ration and by injecting vitamin A–ester intravenously every five days. Vitamin A blood level was an unreliable indicator of vitamin A status. For example, animals that had been on a low carotene intake for three to five years (50 micrograms of carotene per kilogram of body weight) had blood plasma levels in the range of 0.20 to 0.50 parts per million (p.p.m.) and a similar range was found in animals on a normal ration of grass silage, alfalfa hay and irrigated pasture in season. Liver vitamin A concentration was, in general, well below 40 micrograms per gram (fresh weight) in cows that subsisted for prolonged periods on the low carotene ration. Livers of animals on the normal ration contained from 75 to 200 micrograms per gram. Milk levels of vitamin A were in the range of 1 to 2 micrograms per gram in animals on low carotene intake and ranged from 6 to 9 micrograms per gram in animals on the normal ration.

It is surprising that repeated injections of 1,250,000 I.U. of vitamin A ester failed to result in any appreciable increase in liver vitamin A in depleted animals. Increasing the carotene in the ration to 390 micrograms per kilogram of body weight likewise failed to increase the liver level of vitamin A in an animal that had subsisted for five years on the low carotene ration and the liver of which was largely depleted of vitamin A stores (15 micrograms per gram). Cows beginning with normal liver vitamin A values of about 200 micrograms per gram and placed on the depletion ration with a carotene intake of 130 micrograms per kilogram of body weight maintained the high milk vitamin A levels but gradually depleted their liver stores to a level of about 100 micrograms per gram.

Although the study by Byers and associates does not permit broad interpretation because of the limited number of animals in the experiment, the results strongly suggest that liver storage of vitamin A is seriously affected by the previous nutritional status of the animal. Animals conditioned to a continued low carotene intake for long periods apparently lack the ability to effect storage even with massive doses of vitamin A ester. The results suggest that a reevaluation of the requirements, utilization,

conversion and storage of carotene and vitamin A in dairy cattle is desirable. The level of 50 micrograms of carotene per kilogram of body weight daily that was used by Byers and his associates as a low carotene intake is about one-third the level presently recommended for the maintenance of good vitamin A nutritional status in cattle.

ENDOCRINES AND MEAT PRODUCTION

The belief that hormones play a major role in determining the rate of growth, efficiency of feed utilization and quality of meat produced by an animal is widely held. As a result, the custom of castrating male meat-producing animals is practiced in animal husbandry. However, E. Wierbicki and co-workers (*J. Agr. Food Chem.* **3,** *244* (*1955*)) point out that the relation of castration to the efficiency of meat production and the quality of meat has never been critically investigated. In three successive years, they conducted detailed experiments in which the influence of castration was studied. In the third year they also compared the influence of diethylstilbestrol administration on the quantity and quality of the meat produced. Altogether, 114 animals were used in these studies, of which 42 were untreated bulls, 57 were steers and 15 were bulls treated with diethylstilbestrol.

In general, castration was undertaken when the calves were 2 to 6 months of age. All animals were slaughtered between 13 and 15 months of age, at which time careful data were collected on the average live weight, the average weight of the chilled carcass, the dressing percentage, and the edible portion, as well as the percentage of waste fat, bone, head and hide. Determinations were made of the amount of intramuscular fat, and of the total amount of myohemoglobin and hemoglobin in the muscle, and evaluations were made of the carcass grade and tenderness of the meat.

In all three years the bulls gained weight at an appreciably faster rate than did the steers. For example, in the second year of the experiment the average daily gain for the 24 steers was 1.95 lb. as compared to 2.43 for the bulls. The bulls which received one or two diethylstilbestrol implantations during the third year of this experiment grew at still faster rates than did the young bulls. Although the common belief is that the dressing percentage of the carcass to live weight is usually higher for steers than bulls, this was not true in the two experiments when the animals were killed at the same body weight. In the first year when the animals were killed at the same age, the steers did have a slightly higher dressing percentage than did the bulls. In all three years the yield of edible portion of meat was significantly greater for bulls than for steers, whether this was expressed on the live animal or the carcass basis, with the bulls yielding 3 to 4 per cent more meat than the steers. There was less waste fat in the carcasses of the bulls, which further increased their meat yield, even though they had a slightly greater amount of bone, head and hide. However, when the tenderness of the meat from the bulls and steers was estimated, the meat of the bulls graded as slightly more tough than that of the steers. The difference was considered by the investigators to be rather small even though significant.

The authors believe the slight difference in tenderness was not worth the cost of producing it, at least for most consumers. In the standard evaluation of the carcass grade on the basis of established criteria for evaluation of steer meat, the bulls had a somewhat less desirable rating than the steers. One reason for this was the fact that the "marbling" looked for in prime grades of beef did not occur so frequently or extensively in bulls because of

the lower amount of fat throughout the carcass. The investigators believe marbling was not a sign of true tenderness and true carcass quality, but rather an expression of the sex difference.

A slightly higher amount of color as expressed on the basis of the total amount of myoglobin and hemoglobin was observed in the meat from steers than that from bulls. The amount of hydroxyproline which is used as an expression of the total amount of connective tissue was higher for bulls than for steers. It is noteworthy that all the animals in this report had a somewhat lower hydroxyproline content as compared to market animals of unknown history. It was speculated that this was because all the animals in this group were young at the time of slaughter. This would suggest that the amount of connective tissue in these steers and bulls contributed little if anything to the degree of toughness of the meat.

It is a wholesome sign to see an age-old concept such as this being subjected to thorough scientific scrutiny. One question that cannot be answered from the data presented is whether the bulls actually used feed more efficiently to attain the weights reported or whether they ate more feed. The investigators' statement that the animals were fed under comparable conditions does not answer the above query. If feed consumption actually were higher for the bulls, it could well be that the apparent greater feed efficiency of the bulls, as evidenced by more rapid growth, was nonexistent. However, the shorter length of time necessary to bring the bulls to market would in itself be an economy feature to the farmer. Questions might also be asked as to the palatability and nutritional quality of the meat produced. Data on these points would be of interest.

POTENTIALS FOR INCREASED FOOD UTILIZATION FROM AVAILABLE LAND AREAS

New sources of food, more efficient use of arable soil, methods to improve the quality of common foodstuffs, and the extent of utilization of foodstuffs are problems which continue to be of paramount importance in view of the increasing world population. Recently various phases of these problems have been discussed: the production of the alga, *Chlorella*, for food purposes (*Nutrition Reviews* **11,** *77* (*1953*); **12,** *111* (*1954*); **13,** *45* (*1955*)), the commercial production of food yeast from various by-products (*Ibid.* **13,** *144*, *160* (*1955*)), the vast, barely utilized food potential of the sea (*Ibid.* **12,** *72* (*1954*)), improvement of grazing lands (*Ibid.* **13,** *171* (*1955*)), and the development of ways to treat cottonseed products in order that they may be used more extensively in animal feeds (*Ibid.* **13,** *173* (*1955*)).

In a recent survey article, F. J. Weiss (*J. Agr. Food Chem.* **3,** *388* (*1955*)) has presented a more optimistic viewpoint about the ultimate solution of these general problems than is generally provided. He likens the pessimistic views to the estimation of the productive capacity of a factory on the sole basis of its floor space without any consideration of human ingenuity and resourcefulness. To substantiate this contention, he points out that about 400 billion tons of sugar have been estimated to be produced each year as primary products of photosynthesis by land and sea plants (G. M. Smith *et al.*, "A Textbook of General Botany," *5th edition, Macmillan, New York* (*1953*)). Of this enormous amount, 90 per cent is estimated to be synthesized by marine and fresh water algae and the rest by the combined activities of all varieties of land plants. Among the land plants, forests produce about 67.5 per cent of the total photosynthetic products while cultivated plants yield only 24.5 per cent (E. I. Rabinowitch, "Photosynthesis and Related Proc-

esses," *Vol. I, Interscience Publishers, New York (1945)*).

Of the primary photosynthetic products produced by land plants, only 2 per cent are used by man and animals, and only 0.2 per cent of all matter produced annually by land plants is used by the entire human race for food, clothing, fuel and building materials (H. T. Northen, "Introductory Plant Science," *Ronald Press, New York (1953)*). Of course, a high percentage of the plant materials is unavailable for digestive purposes by the human and animal population because the primary products of photosynthesis are used as energy sources by the producing plant or rapidly converted into other plant products. Only a small part of the photosynthetic products remains in the readily utilizable forms of fat, protein and digestible carbohydrates. The extreme example of plant material which is presently unavailable for man or animals to use as food is wood, which contains an average of 60 per cent cellulose, 30 per cent lignin, 10 per cent hemicelluloses and as little as 0.1 to 0.3 per cent nitrogen.

In view of the poor state of nutrition in wide areas of the world and the ever-increasing food needs as the population increases, the fact that man can use such a tiny percentage of the plant material produced annually is a tremendous challenge. The greatest prevalence of deficiency diseases is in tropical areas where the annual production of vegetable matter is the highest in the world. Weiss points out that in areas of tropical rain forests, plant production may be as high as 80 tons per acre per year as compared to only 2.5 tons for forests in the temperate zone and 5 to 6 tons per acre for whole plants of high yield hybrid corn. Even where the average acreage production is many times lower than for the tropical forests, there is a relatively inefficient rate of utilization of the materials produced; yet with this inefficient utilization, national food surpluses in the United States are large.

Examples of the extent of farm residues merit description. Current estimates of the total straw production in the United States are 117.1 million tons. An additional 98.8 million tons of corn stover (mature stalks and leaves after the corn cobs have been removed) are produced and left as farm residues annually. In addition to these "waste" products, there are 43.1 million tons of such residual materials as corn cobs, cotton and soybean stems, and bagasse, the plant structural material left after the juice is expressed from sugar cane. Altogether these farm residues total 259.0 million tons annually, of which approximately 9.0 per cent is water. It is true that much of this material is not a waste product in the narrow sense of the term, for when left on the field it fulfills essential functions in soil building and in providing subsequent crops with organic matter and mineral nutrients. Thus it would be a bad practice if all these residues were removed from the land, even if this were economically feasible. However, Weiss points out there are many instances where crop residues accumulate in such amounts on the farm that they constitute an embarrassment to the farmer. The situation is even more acute at processing plants where it is uneconomical at present to destroy or cart away these farm residues. If only 5 per cent of the total farm residue could be converted into animal feedstuffs with an average of 80 per cent of the original calories in the form of carbohydrates we would obtain an additional 10.4 million tons of farm residues for ruminating animals.

Another form of plant residue is the total wood waste in logging and primary manufacturing operations. In 1944 approximately 108.9 million tons or 43 per cent of the wood cut became waste (R. K. Winters *et al.*, "Wood Wastes in the United States," *U. S. Dept. Agriculture, Forest Service Report No. 4, Washington (1947)*). Approximately 40 per cent of this waste was used as a fuel in a relatively inefficient fashion while the remaining 60 per cent was not used at all. Weiss points out that there is a variety of fungi, the white-rot fungi, which contains lignase and which in the forest

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produces a low lignin, high cellulose product from wood. If a commercially effective strain of white-rot fungi could be found, finely divided woods such as sawdust would offer a favorable substratum for fungal activity. This would be especially true if adequate nutrients containing inorganic nitrogen and traces of minerals were supplied and these nutrients would become integrated with the final product of the fungal metabolism, making the delignified fodder more nutritious for the animals to which it was fed. If 10 per cent of the wood wastes of the United States could be used annually for conversion into animal food in which 65 per cent of the original energy available was utilizable by animals, we would have a new source of 4.3 million tons of carbohydrate fodder from these wood wastes. Thus if 5 per cent of the farm residues and 10 per cent of the wood wastes could be converted into utilizable foodstuffs, we would obtain a total of 14.6 million tons of herbaceous material. This figure may be compared to the 18.8 million tons of commercial feed that were prepared in 1951.

Weiss briefly reviews some of the studies that have been made to determine the extent to which various plant materials can be used in animal feeds. During World War II, wood pulp combined with ammonia as a source of nitrogen was used to overcome an acute fodder shortage in Scandinavian countries. In 1942 alone, about 1 million tons of this combination were used for cattle feed with good results. Since the war, this has been uneconomical due to the availability of commercial feeds and the tremendous demands for pulp and paper. In this country, sugar beet pulp has been treated with anhydrous ammonia to produce a cattle feed with a protein equivalent of 25 per cent. Dehydrated citrus pulp was used in similar fashion to produce a desirable fodder with a protein equivalent of 40 per cent. Urea has also been used as a satisfactory partial replacement for protein in the rations of ruminants (*Nutrition Reviews* **12,** *43 (1954)*). Ammoniated blackstrap molasses has been found to be less satisfactory. Weiss also cites feed tests in which wood molasses proved satisfactory in feeding cattle, sheep, hogs and poultry when mixed with other feeds, as well as the potential use of wood molasses as a raw material for the preparation of yeast for human and animal consumption.

Another area which needs further exploration is the use of individual synthetic amino acids to improve diets and simultaneously to increase the food efficiency of the given diet. The use of synthetic methionine as a supplement of 1 pound per ton to broiler or turkey feeds has become a common practice. This supplement costs about $3 per ton of feed treated, but results in an increased feed efficiency of 10 per cent which, translated into production, is about a $10 extra profit per ton of feed. Sheep may also benefit from methionine supplementation, especially when urea is used as a partial protein supplement. Lysine is found in low concentrations in all vegetable proteins with the exception of the legumes. Since the cereal grains and their products constitute large portions of the diet of various populations in the world, lysine deficiency may be more widespread than is generally recognized. Even though there may not be frank evidence of lysine deficiency, protein utilization is undoubtedly inefficient by reason of the low lysine levels. Recently progress has been made in the synthesis of lysine so that there is real hope that relatively large amounts of this amino acid could become available for supplementation purposes if found useful.

J. G. Harrar (*J. Agr. Food Chem.* **3,** *395 (1955)*) has described one phase of the general technical aid program to increase agricultural production in South American countries. An examination of the situation suggested that only a small fraction of the total potential food production is being realized in Latin America. Certain areas are insufficiently populated, but more importantly, food production is limited by

existing agricultural practices, by systems of communication and transportation, by power supplies, by land-holding patterns and by credit facilities. The greatest limiting factor in food production is the level of soil fertility. Mexico is used as an example to illustrate the increased fertilizer demand during recent years, to compete with depleted soil fertility. Fertilizer consumption was 50,000 tons in 1940 but has risen to approximately 800,000 tons in 1955. Many times this amount will be needed in coming years. Peru and Colombia were given as examples where increasing production of such products as corn, wheat, cotton, sugar cane, potatoes and coffee has been made possible by increased fertilization. Nitrogen and phosphorus fertilizers have been the ones for which most evidence has been collected, but relatively little is known about the trace element requirements.

Modern methods for the control of rust and smuts in the cereal grains and insecticides for the control of insect pests in potatoes have made possible normal density planting for cereals, and the cultivation of potatoes in broad level plains or valleys where the benefits of mechanization can be added to the already dramatic increases in yield. Cotton, banana and legume production have similarly been benefited by the extensive use of insecticides and fungicides.

Weed control has also received considerable attention in Latin America. The fact that so much of the area lies in the tropics and subtropics permits year-round cultivation. Yet this desirable feature results in unusually high competition of weeds with agricultural crops. While improvements are being made in cultural practices to control weeds through deep plowing, disking and frequent cultivation, these physical methods have been effectively joined by chemical control procedures.

With increases in crop yields from these procedures, the farmer is sufficiently benefited financially to permit an interest in mechanization. Harrar points out that this usually begins with the small operator obtaining simple devices. He points out further that revision of land tenure and management procedures are in growing demand locally in the Latin American countries and that the meeting of these problems at the local level is fundamental in the normal progression of the agricultural program beyond its present state.

All these ways to increase food production support Weiss' postulate that food potentials far exceed the latent possibilities of tillable land acreage by reason of man's resourcefulness and inventive ingenuity. Such things as crop genetics, fertilizers, pesticides and herbicides have proven their merit at economically feasible levels. Other areas such as the conversion of farm and forest residues into animal feeds need much research before economically sound procedures can be developed. Inevitably as the pressure of world population increases, such procedures must be found and must be utilizable in economically sound fashion.

TRACE ELEMENTS IN PLANTS AND SOILS

Current interest in the minor or trace elements far exceeds that of the early era of observations when many of these elements were shown to be necessary for plant or animal growth. In a recent study, N. O. Price, W. N. Linkous, and R. W. Engel (*J. Agr. Food Chem.* **3,** *226* (*1955*)) have determined the amount of boron, cobalt, copper, manganese, molybdenum and zinc in samples of six types of forage plants, alfalfa, lespedeza, red clover, ladino clover, timothy and orchard grass, that were grown on as many as four different types of soil in the Piedmont Plateau in Virginia. Samples of soils from various areas in the plateau were also analyzed for these elements. Al-

together 88 soil and 153 plant samples were analyzed. The ultimate purpose of these studies was to determine whether adequate amounts of these minor elements were present in the forage plants to provide for the normal nutriture of foraging animals.

The boron content of these plants varied from an average of 2.3 parts per million (p.p.m.) for orchard grass samples from Penn-Bucks soils to as high as 38.4 p.p.m. for alfalfa grown on the same soils. The legumes routinely had higher values than the other two plants. The boron content of the 4 different kinds of soil varied markedly, with the Penn-Bucks soils being the highest on the average and the Cecil soils the lowest. Within each soil belt there were striking differences in the boron content.

The cobalt content of the forages ranged from a low of 0.15 p.p.m. for the 29 lespedeza samples from Tatum-Nason soils to a high of 0.67 p.p.m. for the 10 red clover samples from Davidson soils. Most of the samples were in the range of 0.1 to 0.5 p.p.m. of cobalt. The legumes alfalfa, ladino clover and red clover, had higher concentrations of cobalt than the nonlegumes. The values for lespedeza samples were closer to the nonlegumes than to the legumes. Just as in the case with boron, the soils varied greatly in cobalt content, with the Davidson soils being higher in this element than the other soil types.

The copper content of the timothy samples was lowest, with an average of 5.1 p.p.m. for those grown on Penn-Bucks soil. The highest values were found in the red clover samples, with an average of 20.0 p.p.m. for those from Penn-Bucks soils. There was no striking difference of copper content among the several soils tested, with low and high values represented in each of the four soil types.

The manganese content was highest in the orchard grass samples from Penn-Bucks soils, with an average of 333 p.p.m.; alfalfa samples from Tatum-Nason soils were lowest, averaging 26.8 p.p.m. The soil belts varied greatly in manganese concentration, with the Tatum-Nason and Cecil soils generally less than 600 p.p.m., whereas the Penn-Bucks and Davidson soils generally exceeded 1000 p.p.m.

The majority of the forage samples contained less than 1 p.p.m. of molybdenum, with no definite trend to high or low averages for any of the six crops or for any of the four soil types.

The zinc content of the forage plants was slightly lower for timothy and orchard grass than for the leguminous plants. Soils of the Davidson belt were somewhat higher in zinc than those of the Penn-Bucks or Cecil belts. However, the Tatum-Nason soils had only about half as much zinc as those of the Davidson type.

When judged by the minimum amounts of trace elements believed to be necessary for animal health, it was evident that only a limited number of the forage plants analyzed in the present study were in the borderline range of deficiency in cobalt and copper. The molybdenum, zinc, manganese and boron concentrations found in the forage samples were within the normal range reported by other workers and were believed to be sufficiently high to provide proper nutrition for grazing animals.

N. R. Page and H. P. Cooper (*J. Agr. Food Chem.* **3,** *222 (1955)*) have studied the effect of solubility of the boron compounds used in fertilizers on the availability of boron to plants. This was studied because crop response to annual applications of boron on the coastal plain soils of the southeastern United States was often highly inconsistent. In addition, boron-sensitive plants were often injured by customary applications of boron-containing fertilizers when certain weather conditions prevailed.

In the first experiment, the investigators compared the analyses of water-soluble boron in four types of soil under three conditions: where no boron was added; where a relatively insoluble boron compound, colemanite, was added in an amount

equivalent to 20 pounds per acre of borax; and where fertilizer borate was used at the same concentration. In all four types of soil it was found that the soluble boron content of the soil at levels at which the roots could utilize the boron was substantially higher for colemanite-treated areas than for fertilizer borate-treated areas. In other studies it was observed that the boron from the relatively soluble fertilizer borate had washed down until it was mostly below the first 18 inches of soil and was unavailable for the plants. It was found that the colemanite had another desirable feature. Immediately after the normal application of fertilizer borate, very high transient levels of soluble boron were found. These levels were sufficiently high to cause toxic effects on boron-sensitive plants. With colemanite applications, similarly high levels were never attained because of the lower solubility.

In another type of experiment, still less soluble boron materials in the form of borosilicates called soft glass frits were used. These are produced by the same general process used to prepare cover-coat or ground-coat porcelain enamels to apply over sheet steel and can be made with or without the addition of other minor elements. The regular raw materials are heated until the mass is completely molten and then dumped into a large volume of cold water to cause rapid cooling. The fritted glass is then ground to the desired particle size. The preparation used in this experiment contained 5 per cent ferric oxide, 2 per cent each of boron oxide, manganese dioxide, cupric oxide and zinc oxide, and 0.1 per cent molybdenum trioxide. The effect of application of colemanite at the rate of 5 pounds per acre and of the fritted material at levels of 50, 100 and 200 pounds per acre was tested with soybeans, which are boron-sensitive. Where no boron was added to the soil, the boron content of soybean leaves was 27 p.p.m. With the colemanite addition, the level in the leaves was increased to 44 p.p.m. However, with the fritted material at 50 and 100 pounds, the levels were only 34 and 37 p.p.m. Not until the 200 pound per acre level was reached did the boron content of the leaves increase beyond the level observed with colemanite to 47 p.p.m. It is interesting to note that the colemanite supplement did not result in an increased yield of soybeans whereas the three levels of frits gave appreciable increases in soybean production, with the best yield at 100 pounds of frits per acre.

These data indicate that it is possible to supply the boron requirements of crops through less soluble boron compounds which will maintain the soluble boron content of the soil at efficient levels for longer periods of time. In addition, the less soluble compounds reduce the possibility of toxicity due to the initial high levels of boron produced in the soil by readily soluble compounds.

FERRITIN BIOSYNTHESIS

Ferritin is a protein containing micelles of ferric hydroxide and ferric phosphate. The substance is widely distributed in the body and is considered to play a major role in the transport and storage of iron. The concentration of ferritin in the mucosa of the small intestine is believed to regulate the absorption of iron from the gastrointestinal tract (*see* S. Granick, *Science* **103,** *107* (*1946*)). In addition to ferritin, apoferritin is present in the various tissues. This compound is the protein moiety of ferritin and contains no iron. When iron is injected into an animal the concentration of ferritin increases. Granick (*Bull. N. Y. Acad. Med.* **25,** *403* (*1949*)) suggested that the iron combines with the apoferritin to form ferritin and that the latter compound is less suscep-

tible to enzymatic degradation than is apoferritin. An alternative explanation would be that the administered iron stimulates the synthesis of apoferritin which in turn would react with iron to form ferritin.

This question of the effect of iron on ferritin synthesis has been elucidated by R. A. Fineberg and D. M. Greenberg (*J. Biol. Chem.* **214,** *97, 107 (1955)*). These workers determined the effect of iron administration on the incorporation of labeled amino acids into ferritin and apoferritin. Guinea pigs were given a diet of natural foodstuffs and one member of a pair was given a dose of 1.5 mg. of iron as ferric ammonium citrate intraperitoneally. Two hours later the control (no injection) and iron-treated animals were given a tracer dose of either DL-leucine hydrochloride-3-C^{14} or glycine-2-C^{14}. The animals were killed four hours later and liver total ferritin (ferritin plus apoferritin) and mixed liver proteins were isolated for C^{14} measurements. Liver total ferritin was isolated by an immunologic procedure. Antiserum was prepared by injecting crystalline ferritin into rabbits. The antiserum was then added to liver homogenates from the treated guinea pigs and the precipitated ferritin-antibody complex was collected and assayed for C^{14}. Mixed liver proteins were obtained by trichloroacetic acid precipitation.

The results were quite clear-cut and were similar with both labeled amino acids. The iron-treated animals incorporated from four to five times as much of the labeled amino acids into total liver ferritin as did the uninjected animals. In contrast, the iron injections did not affect the incorporation of the labeled amino acids into mixed liver protein. The results then show that the increase in ferritin concentration resulting from iron administration is due to an increased rate of synthesis rather than to a decreased rate of degradation.

The next experiments were designed to elucidate the ferritin-apoferritin relationships. It is conceivable that ferritin is synthesized first and that apoferritin is formed by removal of iron from the ferritin molecule. To study the problem, iron-treated animals were killed at various intervals after the injection of the labeled amino acids. The total ferritin, isolated by the immunologic procedure, was subjected to centrifugation at 10,000 times gravity. It was expected that the ferritin with the higher iron content would sediment more rapidly. The iron content of the various fractions supported this hypothesis. As would be expected, it was found that there were several fractions of ferritin with differing iron content and also a fraction consisting chiefly of apoferritin.

When the animals were killed one-half hour after the labeled amino acid was injected it was found that the total ferritin fraction with the lowest iron content exhibited approximately fifty times greater specific activity (C^{14} content) than the fraction with the highest iron content. When the time interval between the injection of the radioactive amino acid and killing of the animal was increased this difference became less marked. These results are exactly what would be expected if apoferritin was acting as the precursor of ferritin.

The results of these experiments add to our knowledge of iron metabolism because they clarify certain details. They show that ferritin is formed by the addition of iron to apoferritin and indicate that iron injection stimulates the synthesis of apoferritin.

CELL CHANGES FROM ANTIMETABOLITES

Mapping the areas of the body which are more or less selectively damaged by various antimetabolic substances may yield valuable information about the metabolism of many tissues. Furthermore, the microscopic changes produced *in vivo* by a metabolic

inhibitor may be compared profitably with the enzymatic changes observed *in vitro*. This type of comparison gives new insight into other factors responsible for cell vulnerability to injury, such as rate of blood flow, the extent of cell permeability, varying rates of detoxification or excretion, the quantities of "reserve" enzyme a given cell has and the effectiveness of alternate routes of metabolism.

Using this approach to the study of antimetabolites S. P. Hicks (*Arch. Path.* **49,** *111* (*1950*); **50,** *545* (*1950*)) has demonstrated certain microscopic similarities in the brain damage produced by sodium azide, malononitrile and cyanide. Each of these compounds produced a similar pattern of selective demyelinization and necrosis in the white matter, possibly related to inhibition of the cytochrome oxidase system, which is essential to metabolism of this tissue, but which is present in low concentration. Thus the cytochrome system may constitute an "enzymatic bottle-neck" for white matter. A similar selective necrosis of cardiac muscle by either fluoroacetate or plasmocid (8-[3-diethylaminopropylamino]-6-methoxy quinoline) suggests that this essential tissue is particularly vulnerable at the converging point of lactate, pyruvate and glucose metabolism.

In 1953 S. P. Hicks (*Arch. Path.* **55,** *302* (*1953*)) extended his observations to embryonic, neonatal and adult rats and mice. He found, as judged by neuronal necrosis, that the neurectoderm was invulnerable to a wide variety of metabolic interruptions, while the embryonic neuroblast was mainly sensitive to substances having "anti-sulfhydryl" action and was quite resistant to anoxia and hypoglycemia. The neonatal brain tissue was little affected by severe anoxia, but quite susceptible to any antimetabolite interrupting glucose utilization, which it apparently can use either aerobically or anaerobically. The adult neurone, as might be expected, was sensitive both to anoxia and to glucose deprivation. Only fluoroacetate and cortisone produced damage to both the neuroblasts and the neonatal neurones, suggesting a high dependency on formation and release of phosphate bond energy.

More recently S. P. Hicks (*Am. J. Path.* **31,** *189* (*1955*)) has described selective lesions in the hypothalamus, peripheral ganglions and adrenal medulla produced by 3-acetylpyridine, an analogue of niacinamide. These lesions are prevented by additional niacinamide given simultaneously (*see also Nutrition Reviews* **13,** *311* (*1955*)). Eleven mg. of the acetylpyridine was found to be lethal within a few hours and when 24 mice were given from 4 to 11 mg. of this substance, either intraperitoneally or subcutaneously, similar lesions were found in all. These consisted of necrosis of the adrenal medulla, of neurons of the spinal and sympathetic ganglions, of the supraoptic nucleus of the hypothalamus, and of the pyramidal layer of the hippocampus. Mice receiving 10 mg. of niacinamide intraperitoneally shortly before being given 11 mg. of acetylpyridine showed no lesions and none died.

Lesions in this part of the nervous system apparently have not been described in mice or rats with severe niacinamide deficiency, and Hicks did not observe them in his control series subjected to prolonged dietary niacinamide deprivation. Nevertheless, the evidence would suggest that these lesions represent a pathologic effect of severe localized interruption of niacin utilization. As a tool for further investigation of the role of niacinamide in cellular metabolism and as a method of producing selective damage to the sympathetic nervous system and adrenal medulla, 3-acetylpyridine would appear to deserve further study.

Selective damage of other tissues resulting from administration of DL-ethionine, an analogue of methionine, has been reported by W. E. Loring and L. J. Hartley (*Am. J. Path.* **31,** *521* (*1955*)). Rats of both sexes were given repeated intraperitoneal or intravenous injections of DL-ethionine daily or

on alternate days while receiving a standard laboratory diet. The total dosage ranged from 12.5 to 900 mg. and the animals were autopsied eight hours to forty-one days after the initiation of the injections.

These workers observed minimal fatty changes in the hepatic cells and little pancreatic acinar destruction, in contrast to the findings of previous investigators, a fact which they relate to the short recovery periods allowed by the closely spaced injections. Of considerable interest was the finding of destruction of the chief cells of the gastric mucosa, dissolution of the epithelium of the smaller ducts and some of the acini of the salivary glands, and similar changes of varying severity in the duodenal epithelium and glands. Since these changes have not been described in previous pathologic studies of ethionine administration they must await confirmation, but they suggest a new area of interest in this methionine inhibitor. It would appear that many of the cells involved in elaborating digestive enzymes are similarly damaged by ethionine and that a similar methionine metabolism of these cells may be important in maintaining their integrity.

AVAILABILITY OF VITAMIN B_6 (PYRIDOXINE) IN HEATED MILK

Vitamin B_6 (pyridoxine) deficiency has only recently been observed in man. The disease has been produced in human subjects by a deficient diet (S. E. Snyderman, L. E. Holt, R. Carretero, and K. Jacobs, *J. Clin. Nutrition* **1,** *200* (*1953*)) and also by the administration of an antimetabolite of pyridoxine (R. W. Vilter and co-workers, *J. Lab. Clin. Med.* **42,** *335* (*1953*)).

Following the observation that convulsive seizures in infants could be alleviated by pyridoxine administration (D. B. Coursin, *J. Am. Med. Assn.* **154,** *406* (*1954*)), it became evident that the heat processing during the preparation of canned liquid milk products resulted in some destruction of pyridoxine. Sterilized milk products of the type used in infant formulas were found to contain 33 to 64 per cent of the vitamin B_6 activity of fresh milk when the assay was done microbiologically with *S. carlsbergensis* as the test organism (J. B. Hassinen, G. T. Durbin, and F. W. Bernhart, *J. Nutrition* **53,** *249* (*1954*)).

In a recent study reported by R. M. Tomarelli, E. R. Spence, and F. W. Bernhart (*J. Agr. Food Chem.* **3,** *338* (*1955*)) it was found that the microbiologic assay for pyridoxine is not a proper assessment of its activity or value in processed milk for higher animals. These workers used the rat assay patterned after that of P. S. Sarma, E. E. Snell, and C. A. Elvehjem (*J. Biol. Chem.* **165,** *55* (*1946*)). Weanling rats were partially depleted of vitamin B_6 by feeding a purified diet for two weeks. The rats were then either continued on the basal diet or received varying amounts of vitamin B_6 in the form of pyridoxine hydrochloride, or heated or unheated milk, added to the basal diet. The basal diet used was formulated to simulate milk with the basic constituents consisting of 375 g. of lactose, 258 g. of casein, and 297 g. of butter oil. This mixture was supplemented with adequate amounts of vitamins other than pyridoxine and with minerals.

The growth response of pyridoxine-depleted rats indicated that canned evaporated milk had only about one-half the pyridoxine content found by microbiologic assay. For example, 22 and 23 g. of heated milk solids supplied 60 micrograms of microbiologically active pyridoxine, but had a biologic activity for the rat of only about 30 micrograms when compared with responses to crystalline pyridoxine hydrochloride or pyridoxal phosphate.

Spray-dried fresh milk used in these studies gave rat responses essentially in agreement with the responses obtained by microbiologic assay. This particular product contained from 3.8 to 4.1 micrograms of pyridoxine per gram. It is pertinent to note that the pyridoxine deficiency syndrome has not been observed in infants fed formulas marketed as a spray-dried powder (P. György, *J. Clin. Nutrition* **2,** *44 (1954)*).

Tomarelli and his associates do not have an explanation for the decrease in biologic activity of pyridoxine as a result of the heat sterilization of milk. They point out that the vitamin may be converted to a form that still permits microorganism usage but limits usage for the rat. It is also possible that the heat treatment may modify other milk constituents to the extent that the pyridoxine requirement could be influenced in an indirect manner. It appears, for example, that highly processed proteins are involved in the increased requirement for pyridoxine by rats when they are fed combat rations (*Nutrition Reviews* **12,** *184 (1954)*). This observation, and the presently noted effects in heat sterilization of milk, suggest that heat processing may have the general consequence of elevating dietary pyridoxine requirement. The nature of the mechanism involved is unknown.

Present information on the pyridoxine content of human milk indicates a level of about 0.15 mg. per liter. Infant formulas prepared from diluted and heat sterilized cow's milk contain between 0.10 and 0.23 mg. per liter. Hence the content of the formulas compares favorably with human milk when they are judged as to microbiologically available pyridoxine. The results of Tomarelli and associates indicate that this assay is not adequate for the proper assessment of the available pyridoxine in the infant formulas.

SYNTHESIS AND DESTRUCTION OF VITAMIN B_{12} BY TUMORS

In a recent report, D. W. Woolley (*Proc. Nat. Acad. Sci.* **39,** *6 (1953)*; *Nutrition Reviews* **11,** *243 (1953)*) presented evidence indicating that in female Swiss mice bearing spontaneous mammary cancers the ability to synthesize vitamin B_{12} is greatly enhanced. The measure of the rate of synthesis employed was the growth rate on a vitamin B_{12}-deficient diet of the young of mothers which had been maintained on the same diet for two or more weeks.

This work has now been extended (D. W. Woolley, *Proc. Nat. Acad. Sci.* **41,** *111 (1955)*) with the use of a less equivocal analytic method for the vitamin. Microbiologic assay (by a chrysomonad) of homogenized, digested and autoclaved mice indicated a mean vitamin B_{12} content of 9 millimicrograms per gram of normal mouse after two and one-half weeks on the deficient diet. For mice on the same diet but bearing spontaneous mammary tumors, the value was 17.2 millimicrograms per gram, including the tumor. When the tumor and the remainder of the carcass were assayed separately, the values were 15 and 30 millimicrograms per gram respectively. This difference was shown later to be due in part to destruction of the vitamin by the tumor.

In the previous report it was stated that the tumor lost its ability to promote the synthesis of the vitamin following transplantation. The present paper, however, reports synthesis of the vitamin by the fourth to eighth passages of transplanted mammary cancer (strain W 137). Another transplanted strain (W 113), however, not only gave no evidence for synthesis of the vitamin but may actually have promoted its destruction since mice with these tumors contained less vitamin B_{12} after two weeks on the deficient diet than did the controls.

Thus when animals bearing this tumor were analyzed without removing the tumor, the average vitamin B_{12} content was 2.1 millimicrograms per gram as compared with 9 for the controls. In separate experiments, destruction of vitamin B_{12} by minced tumor tissue took place during incubation for one hour at 37°C. Of four types of tumors used, three gave evidence for both synthesis and destruction, while one (W 113) showed only destruction.

The author was unable to conclude from his experiments whether actual synthesis of the vitamin took place in the tumor-bearing animals or whether the excess vitamin was present because of increased storage. An experiment designed to settle this question gave an equivocal answer, since mice which had been on a vitamin B_{12}-deficient diet for two weeks before transplantation of the tumor contained somewhat more vitamin (after a further two to four weeks on the diet) than did the controls (15.5 millimicrograms versus 10.0), but not to the extent found in previous experiments.

Finally, some evidence was gained during the course of these experiments in favor of the general hypothesis which prompted the research (D. W. Woolley and A. Pringle, *J. Biol. Chem.* **194,** *729 (1952)*)—that those tumors possessing the ability to synthesize a vitamin should be susceptible to the action of an antimetabolite analogue to a precursor of the vitamin. Transplanted strain 137, which was the most susceptible to the action of the antimetabolites of 1,2-dimethyl-4,5-diaminobenzene also showed the best evidence for synthesis of vitamin B_{12} by a transplanted tumor. This type of approach, involving a study of the nutritional requirements of the neoplasm in relationship to those of the host, should offer a worthwhile and logical approach to the chemotherapy of cancer.

AMINO ACID REQUIREMENTS OF MAMMALIAN CELLS IN TISSUE CULTURE

The culture of mammalian cells *in vitro* has been the subject of investigations for almost half a century (*Nutrition Reviews* **5,** *189 (1947)*). The advantages of attaining knowledge of nutrition through this approach have long been recognized. With a chemically defined medium it would be theoretically possible to determine the nutritional requirements of each individual type of body cell, which would thus permit better delineation of relationships between the different types of cells in the living organism. These researches have been beset by technical difficulties, and culture of cells on a "synthetic" medium for an indefinite time has not been achieved.

Adequacy of the medium and essentiality of added nutrients have been assessed by their effect on length of survival time of the cells (*Nutrition Reviews* **8,** *181 (1950)*). Attempts to establish amino acid requirements have been made by supplementation of mediums containing dialyzed serum, plasma, or embryo extract in fairly large amounts; however, removal of individual amino acids did not result in the clear-cut effects expected on the growth of the culture (*Ibid.* **7,** *8 (1949)*; A. Fischer, *Biochem. J.* **43,** *491 (1948)*).

More recently, H. Eagle (*J. Biol. Chem.* **214,** *839 (1955)*) has determined the amino acid requirements of a culture of adult mouse fibroblasts (strain L) in a synthetic medium containing very small amounts of dialyzed horse serum. In addition, characteristic signs of deficiency were seen when any required amino acid was omitted from the medium.

In these experiments, the cells were grown in glass flasks under standardized conditions. The cells of an inoculum sedimented, attached themselves to the glass, and grew as a monocellular layer. The basal medium contained nineteen amino acids, fifteen vita-

mins, seven salts, five other growth factors, and 0.25 to 2 per cent dialyzed horse serum. Approximately half of the components of the medium were eventually found to be nonessential. In the absence of the small amount of horse serum, cells failed to grow normally and survive; however, cultures were maintained with no apparent changes for periods of four to eight weeks in this medium containing dialyzed serum.

By the omission of single amino acids from the medium, a requirement was established for twelve amino acids. These were arginine, cysteine (or cystine), histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine. It is of interest that in addition to the amino acids required by the intact animal, the cell also required arginine, cysteine and tyrosine. Omission of a single essential amino acid from the medium resulted in cessation of growth accompanied by marked alterations in structure. The cytopathologic effects were completely reversible within one to three days if the missing amino acid was supplied between the second and sixth day of deficiency.

The requirements of the individual amino acids for maximal growth ranged from 0.005 micromoles per milliliter of medium for tryptophan to 0.1 to 0.2 micromoles per milliliter for isoleucine. Increasing the amino acid level above the requirement for maximal growth caused a rapid development of toxicity with decline in growth in the case of methionine and tryptophan. Methionine is also one of the most toxic amino acids for animals. Less pronounced evidence of toxicity for the culture was seen with isoleucine and phenylalanine, whereas increasing the concentration of any of the other essential amino acids by fifty to 500 times had no deleterious effect. For all of the amino acids, only the L-forms were active in supporting growth of the tissue culture.

The rate of growth was much faster in tissue culture than in the animal, which may have some bearing on the greater number of amino acids required by the tissue culture than by the intact mouse. The cultured cells had markedly different morphologic characteristics from the parent cells in the animal. It appears possible that the structural changes may represent reversion to a simpler type of cell, possibly embryonic in nature. If this were true, then it is not inconceivable that the requirements of a variety of cells might be the same in tissue culture.

In order for findings obtained *in vitro* to be of significance in interpreting studies on the whole animal, it will be necessary to show that the functional capacities (*i.e.*, metabolic pathways) of the cells are similar under the two sets of circumstances. In view of the presently known differences in cell growth *in vitro* versus *in vivo* this is perhaps another situation where experimentally "the whole does not equal the sum of its parts" (statement attributed to P. W. Bridgman).

The work of Eagle represents a considerable advance in understanding of the nutrition of a single type of cell grown *in vitro*. It is a substantial contribution to the science and the art of tissue culture, which may have significance in studies on pharmacology and chemotherapy. How accurately information obtained by this technic can be applied to the same cell growing *in vivo* is not known; however, the time is approaching when it will be possible to appraise the problem more critically.

PROTEIN FEEDING AND WOUND HEALING

In recent years there has been considerable interest in the metabolic abnormalities following acute trauma. An increased urinary excretion of nitrogenous products following

injury, major operations and serious acute illnesses has been observed by many investigators. Several factors, including reduced food intake, fever, bed rest, endocrine imbalance, increased oxygen consumption, and breakdown of injured tissue, may influence the extent of the increase in urinary nitrogen excretion (*Nutrition Reviews* **10,** *195 (1952)*; **12,** *328 (1954)*). Few objective data concerning the physiologic and clinical sequelae directly attributable to the early "catabolic" reaction to injury are available. There are conflicting opinions as to whether the catabolic phase is harmful and, depending on the viewpoint taken, whether attempts should be made to modify the process. Much of the conflict is due to the lack of objective indices for measuring the influence of this change in metabolism.

D. H. Calloway, M. I. Grossman, J. Bowman, and W. K. Calhoun (*Surgery* **37,** *935 (1955)*) have compared the nitrogen balance and the healing of sutured incisional wounds of rats fed minimal or liberal levels of protein preceding and following injury. Male albino rats, 250 to 300 g. in weight, were the experimental animals. For two weeks preceding wounding all rats were offered diets providing about 48 calories daily but differing in protein content. The rats were divided into three groups receiving one, two, or three times the presumed minimum requirement for nitrogen equilibrium (53 mg. of nitrogen per 100 g. of body weight per day). Casein supplemented with methionine, 30 mg. per gram of protein, was the protein source. The differences in protein contents were compensated calorically with sucrose. Fat supplied about 15 per cent of the total caloric intake in each group.

Midline laparotomy incisions, 4 cm. in length, were made in rats anesthetized with sodium pentobarbital. The wounds were closed with interrupted through-and-through sutures of fine stainless steel. Immediately thereafter, a shaved area on the back was burned by hot water (90° C.) for 15 seconds. The burns involved about 6 per cent of the rat's body surface.

On the day of operation and burning, water was offered ad libitum, but no food was given. Two thirds of the preoperative food intake was offered on the first postoperative day. Thereafter, the groups prefed one or three times the protein requirement were divided, one half of each group continuing on the preoperative diet, the other half receiving twice the normal minimal protein requirement. In this way there were three groups which differed only in the diet fed before injury and three groups in which the preoperative and postoperative diets were the same, but fed at three different levels.

Five or 6 animals from each group were killed on the fourth, seventh and tenth postinjury days. In addition, 5 animals of each of the three preoperative groups were killed after the two-week control period without being subjected to any injury.

During the control periods all animals (as measured on the last two days of the period) were in positive nitrogen balance, about 70 to 80 mg. of nitrogen per day. Urinary nitrogen excretions and, thereby, negative nitrogen balances were less on the day of injury and the first postoperative day in the groups prefed the lower amount of proteins. This would be expected as a result of the drastic reduction of food intake on the operative day and the moderate reduction on the next day. Thereafter, the animals fed the higher protein intakes postoperatively were in more positive nitrogen balance than those fed the minimal protein levels, but there was no significant difference between those groups receiving two or three times the minimal protein levels. The periods of negative nitrogen balance were very brief.

Liver nitrogen content was higher at the end of the two-week control period and at each sacrifice day (fourth, seventh and tenth) after injury in the rats on the higher

protein intake levels. However, there was little difference between the groups receiving two or three times the minimal levels. Liver fat content was about the same for all groups. The adrenal weights were heavier after injury in all groups; there were no apparent differences among the various groups.

Examination of the abdominal wounds by various histologic technics and tensile strength measurements revealed no apparent differences among the various groups. Wound healing seemed to progress satisfactorily, and at about the same rate, in all instances. The authors conclude that maintenance of a high plane of protein intake before injury is without obvious benefit to rats in terms of wound healing as compared with maintenance of minimal adequate protein intake. Their data suggest a similar conclusion in regard to postoperative dietary levels.

About twenty-five years ago, S. C. Harvey and E. L. Howes (*Ann. Surg.* **91,** *641 (1930)*) compared the healing of stomach wounds in adult rats on a standard Sherman diet with that in adult rats on a high protein diet. The composition of the standard diet in terms of calories was as follows: protein 13.8 per cent, carbohydrate 39.2 per cent, fat 47.0 per cent. The high protein diet consisted of protein 68.7 per cent, fat 31.3 per cent (80 per cent casein, 12 per cent hydrogenated vegetable oil by weight). The animals were kept on the diet one week preoperatively, fasted for six hours preoperatively, but postoperatively feeding was resumed as soon as possible. The caloric intake was slightly less in the animals on the high protein diet.

Harvey and Howes observed no effect of the high protein diet on the lag period but noted an increase in the rate of development of tensile strength of the wounds during the period of five to nine days postoperatively. The high protein diet also shortened the time required to reach the maximum breaking strength, although the ultimate maximum breaking strength was approximately equal in the two groups. No histologic observations of the healing wounds were reported, but in previous studies Harvey and Howes had observed a very close parallelism between fibroplasia, production of the intercellular substances and tensile strength.

The diets employed by Harvey and Howes were higher in fat content than those used by Calloway and her associates. In addition, the fat content of all the diets used by the latter investigators was constant, whereas it was higher in the lower protein diet used by Harvey and Howes. Further, the casein used by Calloway was supplemented with methionine. The importance of methionine in wound healing in rats has been demonstrated by several authors (*Nutrition Reviews* **11,** *124 (1953)*; **13,** *218 (1955)*). However, it is not certain that the lower protein diet employed by Harvey and Howes was inadequate in methionine.

It should be emphasized that the periods of negative nitrogen balances observed in the rats studied by Calloway and her associates were very brief, lasting only one or two days after injury and operation. This is in marked contrast to what is seen following serious injury to patients maintained on dietary intakes normal for healthy adults. Accordingly, no definite conclusion can be made from this study as to the physiologic significance of the metabolic derangement following injury and its modification by dietary means. In rats with much larger burns (30 to 35 per cent of the body surface), S. M. Levenson, C. L. Pirani, J. W. Braash, and D. F. Waterman (*Surg. Gynec. Obstet.* **99,** *74 (1954)*) found that the healing of experimental laparotomy wounds was significantly different from that in unburned controls. Epithelization was not affected, but there was a definite delay in the formation of granulation tissue in the incisional wounds of the burned animals, with a lag in the appearance and maturation of the

fibroblasts and the ground substances. The eventual number and amount of these two elements, however, did not appear to be affected, and abundant granulation formed in the wounds of the burned rats in time. Unfortunately, no measurements of nitrogen balance, or other metabolic changes, were made in this study.

SQUALENE, AN ESSENTIAL NUTRIENT?

During the past few years intensive research by several groups of workers has indicated that cholesterol is synthesized in its entirety from acetate and that squalene is a very probable intermediate in this synthesis (*Nutrition Reviews* **12,** *174 (1954)*; **13,** *160 (1955)*). It is surprising, therefore, to find squalene postulated as an essential nutrient for the maintenance of successful lactation in the albino rat. A few years ago two papers appeared (M. S. El Ridi and M. Shahat, *J. Roy. Egypt. Med. Assn.* **27,** *199 (1944)*; El Ridi, W. M. Azouz, and A. Abdel Hay, *Z. f. physiol. Chem.* **286,** *256 1950)*) which reported that rats on a presumably adequate diet were unable to suckle their young. Addition to the diet of the oily extract of *Foenum graecum* seeds or yeast improved the ability to lactate. The active fraction in these substances was reported to be squalene, which was characterized by the melting point and elemental analysis of the hexachloride.

Confirmation of this hypothesis has now been obtained in several extensive experiments (M. S. El Ridi, W. M. Azouz, and A. Abdel Hay, *Z. f, physiol. Chem.* **299,** *283 (1955)*). The basic diet used in the experiments consisted of cane sugar 65 per cent, casein 20 per cent, lard (squalene content about 4 mg. per cent) 9 per cent, salt mixture 4 per cent, water-soluble vitamins 1 per cent and fat-soluble vitamins 1 per cent. In the experimental group this diet was supplemented with squalene obtained from shark-liver oil. All animals were raised on this diet from weaning and showed normal growth and reproduction. The females were bred when they had attained a weight of 160 to 180 g. They were continued on their respective diets throughout the experiment, food being offered twice daily and then removed. Only up to 6 young from each litter were left with each mother and they were weaned on the twenty-first day. The females were bred again after one week's rest.

On a normal diet, 44 out of 50 mothers bore 330 young, of which 241 were left with the mother and 229 were suckled. The "lactation index" of this group was therefore 95 per cent. On the artificial diet, however, 63 out of 67 animals bore 493 young, of which 393 were left with the mother and 110 were suckled, for a "lactation index" of 28 per cent. In a separate experiment, 10 animals on the artificial diet showed a "lactation index" of 34.9 per cent while a similar number of rats which were given a 10 mg. supplement of squalene daily had an index of 92.1 per cent.

The authors draw attention to the presence of squalene in all the fats judged adequate for animal diets by H. J. Deuel, E. Movitt, and L. F. Hallman (*J. Nutrition* **27,** *509 (1944)*), but admit that much research is needed on the lactation process in general. The obvious question which arises is how squalene can act as an essential nutrient when it is well known that it is synthesized in the animal body entirely from acetate (for a review of the subject *see* R. G. Langdon, "Fat Metabolism, A Symposium," edited by V. A. Najjar, *Chapt. 11, pp. 162–81, The Johns Hopkins Press, Baltimore (1954)*).

It might be suspected that the authors were dealing with essential fatty acid de-

ficiency since their diet would fall in the low fat class. However, this amount of lard undoubtedly contains adequate quantities of the polyunsaturated fatty acids (*see, for example,* L. N. Norcia and W. O. Lundberg, *J. Nutrition* **54,** *509 (1954)*; *Nutrition Reviews* **13,** *214 (1955)*). Furthermore, the rats which had been on this diet since weaning showed none of the more obvious symptoms of fat deficiency disease such as poor growth rate or infertility.

At present the only postulated function of squalene in the animal organism is as an intermediate in cholesterol biosynthesis. In this connection, a recent report by J. Raulin (*Arch. sci. physiol.* **8,** *1 (1954)*; *Chem. Abstr.* **48,** *8892h (1954)*) is of interest. Female rats on a diet similar to that used by El Ridi and co-workers, and in which lard was the sole lipid constituent, again showed poor lactation. In this case, however, lactation was improved by cholesterol feeding.

The designation of a nutrient as essential usually derives from experiments in which the animal in an otherwise normal state is deprived of one specific substance. However, this does not mean that the animal can synthesize all other materials in unlimited quantities. Under stress conditions, in which some normally nonessential substance is rapidly used or destroyed, the capacity of the body for its synthesis may become inadequate and it must then be obtained from the diet. The present work indicates that squalene may be in this class, possibly by virtue of its role in cholesterol biosynthesis.

FLAVONES AND FROSTBITE

Just prior to World War II, it was shown that rapid warming of frostbitten parts of the body is beneficial in preventing permanent injury. (For references *see* F. A. Fuhrman and J. M. Crismon, *J. Clin. Invest.* **26,** *476 (1947)*). The above observation did not deter those who were attempting to determine whether diet or dietary supplements play any role in the prevention of permanent injury following frostbite. This work was stimulated by the large number of frostbite casualties during the Korean conflict.

Recently considerable work has been done on the effect of the flavones in alleviating frostbite injury. F. A. Fuhrman and J. M. Crismon (*J. Clin. Invest.* **27,** *364 (1948)*) reported that gangrene could be prevented in the feet of rabbits exposed to cold when 50 to 100 mg. of rutin (one of the flavones) per kilogram of body weight was given by stomach tube starting on the day of exposure. Rutin, however, had no effect in preventing the development of gangrene in the ears of rabbits exposed to severe cold. Support of these findings was reported by A. M. Ambrose, D. J. Robbins, and F. DeEds (*Fed. Proc.* **9,** *254 (1950)*), whereas R. B. Lewis and P. W. Moen (*Exp. Med. Surg.* **11,** *9 (1953)*) reported that rutin was ineffective in reducing muscle or skin necrosis of rabbits' feet exposed to a freezing bath.

Even in those cases where rutin was reported effective in decreasing injury following frostbite, rapid warming of the frozen extremity afforded better protection than the dietary supplement (F. A. Fuhrman and J. M. Crismon, *J. Clin. Invest.* **26,** *476 (1947)*). In spite of this, work has continued in an effort to evaluate the effectiveness of dietary supplements in this type of injury. One of the latest studies of this nature is that of Fuhrman (*Am. J. Physiol.* **181,** *123 (1955)*), who fed rats a purified diet composed of sucrose, casein, corn oil, salts, and adequate amounts of those vitamins required by the rat. One half of the animals, initially weighing 100 g.,

were fed this diet for six to thirteen days, while the other half received the same diet supplemented with two different flavones (hesperidin methyl chalcone or calcium flavonate glycoside lemon). The supplements were fed at levels of 1 or 2 g. per kilogram of diet for the first compound and 10 g. per kilogram of diet for the second.

At the end of the dietary period, the rats were anesthetized. One foot was immersed to the ankle in a mixture of alcohol and dry ice (−25° C.) until signs of frostbite appeared. Upon removal from the freezing bath, the extremity was permitted to thaw in air. The animals receiving hesperidin showed less tissue loss than the control animals in three series of experiments. The differences between the animals receiving the flavone and the control animals were highly significant in two experiments and of questionable significance in one. The calcium flavonate was used in only one series, where it produced a questionable improvement in the recovery from frostbite. The scoring of the results was made two weeks after the animals were exposed to the cold.

The development of gangrene in frostbitten areas appears to be associated with the development of stasis in the capillary bed (Fuhrman, *loc. cit.*). It was suggested that the mode of action of the flavones in many cases can be explained by their potentiation of epinephrin, with a consequent improvement of blood flow. On this basis, the flavones are postulated as preventing vascular occlusion from becoming extensive enough to produce gangrene. The improved circulation might thus result in a reduction of fluid loss through the capillary walls. It has been stated that the edema fluid from frostbitten extremities contains a vasodepressor substance resembling ferritin. The action of this ferritin-like material can supposedly be counteracted by flavones (J. M. Crismon, R. R. Berez, J. D. Madden, and F. A. Fuhrman, *Am. J. Physiol.* **164,** *391 (1951)*).

These studies on the action of flavones in counteracting frostbite injury are of interest, since they again raise the question of the value and necessity of these compounds in the diet. Although the preceding work suggests a positive action of the flavones in lessening permanent damage following frostbite, the results of the earlier reports augur against a hasty conclusion. There is a possibility that further work in the above area will show a preponderance of negative results similar to the studies on the relation between flavones and radiation injury (*Nutrition Reviews* **10,** *242 (1952)*).

NOTES

Nitrogen Metabolism Versus Intake

Cushing's syndrome, in addition to other clinical findings, is characterized by wasting of muscle mass presumed to be due to an antianabolic effect on protein metabolism of the excess quantities of cortical hormones secreted (*Nutrition Reviews* **11,** *38* (*1953*)). Further studies by K. R. Crispell, W. Parson, and G. Harden (*J. Clin. Invest.* **33,** *342* (*1954*)), again using N^{15}-glycine, have substantiated this conclusion and supplied data on the fate of ingested protein in normal individuals.

Eight healthy volunteers and 1 patient with Cushing's syndrome were studied for nitrogen balance (assuming fecal nitrogen to be 1.3 g. daily). N^{15}-glycine was administered orally or intravenously. Three diets were used, one furnishing daily at least 1.6, a second a maximum of 0.4, and a third approximately 1.0 g. of protein per kilogram of body weight. The amount of N^{15} excreted was determined daily. As in their previous work, the authors found that, although both the normal subjects and the patient with Cushing's syndrome were in approximate nitrogen equilibrium, the excretion of N^{15} increased greatly over the normal after N^{15}-glycine administration in the patient with Cushing's disease. Thus, approximately 26 per cent of the administered N^{15} was excreted by the normal subjects in twenty-four hours while nearly half was excreted by the patient with Cushing's syndrome.

These results were obtained on a normal diet. The low protein diet given to the normal individuals caused a marked decrease in the excretion of N^{15} from the glycine whereas the high protein diet increased the excretion. The patient with Cushing's syndrome given a high protein diet excreted essentially the same percentage of the N^{15} administered as she had on a normal diet.

The authors interpret these results to indicate that in normal individuals increase in dietary protein leads directly to increased urea formation from the excess protein administered whereas on the low protein diet they were unable to differentiate between decreased urea formation or increased synthesis of administered amino acids into tissue protein. It would seem that the former possibility was the more likely. Finally, these workers believe that the failure of a patient with Cushing's syndrome to increase the excretion of N^{15} in changing from a normal to a high protein diet suggested that direct urea formation from ingested amino acids was maximal on the normal protein diet and could not be further increased.

Sodium-Restricted Diets

The wide popularity, clinical usefulness, and many misunderstandings of sodium-restricted diets prompted the National Research Council Food and Nutrition Board to appoint "a committee to review the evidence on the efficacy of sodium-restricted diets for therapeutic purposes and to prepare a report which would summarize the clinical and dietary conditions for their use." The report, jointly sponsored by the Council on Foods and Nutrition of the American Medical Association, has been published ("Sodium-Restricted Diets: The Rationale, Complications, and Practical Aspects of Their Use," *National Academy of Sciences—National Research Council Publication 325, July, 1954*). In addition, three sections of most interest to physicians have been taken from the report and published by the Council on Foods and Nutrition (*J. Am. Med. Assn.* **156,** *1081, 1171, 1252* (*1954*)).

The final report includes an explanation of terms, particularly emphasizing the interconversion of milligrams and milliequivalents. The reporting of the sodium content of the food or diet as sodium rather than as

sodium chloride or as some other compound is also emphasized. A brief summary of our knowledge of the normal physiology of sodium metabolism is included which is followed by an analysis of the clinical usefulness of sodium-restricted diets in such diseases as congestive heart failure, hypertension, renal disease, cirrhosis of the liver, the toxemias of pregnancy, Menière's disease, and during hormone therapy (especially of the adrenal cortical hormones). Emphasis is placed in a third section upon the complications and contraindications to sodium restriction. It is concluded that although a possible hazard exists there is little likelihood that even a severely sodium-restricted diet will compromise adequate nutrition. The possibility of inducing the "sodium depletion syndrome" is emphasized and discussed, as are the metabolic alterations observed during sodium restriction.

The fourth section devoted to planning sodium-restricted diets is built around a basic 500 mg. sodium diet which, with suitable simple alterations, can be more or less restricted in sodium or changed in the content of protein and of other nutrients.

A section devoted to discussion of the sources of sodium discusses not only the sodium content of foods and the effect of cooking, but also drinking water as a source of sodium and some other incidental sources.

Finally, a critical analysis is made of the methods for determining sodium content of foods.

Extensive tabular material includes a complete table of the presently known sodium (and potassium) content of foods (including some new data from the committee), with maximum, minimum and average values for sodium and, when known, the number of samples analyzed. This table illustrates among other things the great variation in the sodium content of individual foods, particularly after different methods of preparation. Clearly, much more analytic data must be obtained before reliable values for the sodium content of foods can be authoritatively given. In spite of this the committee constructed from the first table a second and much shorter table giving what they considered the best estimates of the sodium content of foods. Tabular material on the sodium content of waters and the effect of cooking is included.

The report emphasizes that as physicians, dietitians and patients come to recognize more clearly the sources of sodium in foods and as more foods are manufactured with reduced or very low sodium content, the degree of sodium restriction may become increased. This should permit fewer therapeutic failures, but also more complications may ensue because of the increased effectiveness of sodium restriction. It is pointed out, however, that, as with most therapeutic measures in medicine, the physician must carefully weigh the good to be achieved against possible harmful effects.

Nutritional Status of Mothers and Their Infants

The Children's Fund of Michigan has published a monograph under the above title giving detailed information on the studies by Dr. Icie Macy Hoobler and her co-workers which were reviewed in the January 1955 number of *Nutrition Reviews* (**13,** *5 (1955)*) under the title "Physiologic Adaptation in Pregnancy." There are discussions of how subjects were chosen, diet evaluations, and the biochemical methods used, as well as their results. An extensive bibliography on the chief factors considered in this study of physiologic changes throughout the course of uncomplicated pregnancy is given.

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